

## Synthesis of silver nanoparticles from coffee parasite leaf extract (*Scurrulla ferruginea* (Roxb. ex Jack) Danser) and potential activity as an antioxidant and anticancer

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### ABSTRACT

This study aim is to know the results of the synthesis of silver nanoparticles from quercetin and *Scurrulla ferruginea* (Roxb. Ex Jack) danser as well as its potential as an antioxidant and anticancer. The solvent used in the maceration extraction method in this research is the polar solvent methanol. Isolation and purification of thick extract compounds from coffee parasite leaves were carried out using liquid vacuum chromatography (CVC) and gravity column chromatography (GCC) methods. Silver (Ag) nanoparticle is made with the stirrer method at a speed of 1500 rpm for 60 minutes at a temperature of 70° C. Silver nanoparticle solution was produced and then characterized using FTIR (Fourier Transform Infra Red) and PSA (Particles Size Analyzer). PSA results analysis using extract coffee parasite size 214 nm particles, whereas using flavonoid isolate quercetin confirmed size 29 nm. FTIR showed that there was a stretching vibration of the OH group at a wave number of 3229.25 cm<sup>-1</sup>, the C=O function at the wave number 1637.87 cm<sup>-1</sup>, and the CO functional group at the wave number 1016.31 cm<sup>-1</sup>. Antioxidant test using the DPPH method was carried out in silver nanoparticle solution using extract *Scurrulla ferruginea* (Roxb. Ex Jack) danser and with quercetin obtained an IC<sub>50</sub> value of around 115.4 and 114.9 this result describes as moderate level of antioxidants. Even though the nanoparticle anticancer test was very weak, overall, the synthesis of silver nanoparticles using flavonoid isolates was better than using extracts from coffee parasite leaves.

### Introduction

Chemotherapy is a method of cancer treatment that refers to the use of chemicals to block the growth or kill cancer cells (Yan et al., 2020). However, conventional cancer treatments such as chemotherapy or surgery have limitations related to drug toxicity, unexpected side effects, drug resistance issues, and lack of specificity. Silver nanoparticles overcome these drawbacks by reducing side effects and increasing the efficiency of cancer therapy. One of its distinguishing features is the ability to cross multiple biological barriers and to provide targeted drug delivery (Kajani et al., 2016).

In silver nanoparticle synthesis, excessive chemicals are required which can cause environmental pollution, and require large costs to manufacture. Therefore, the synthesis of nanoparticles using plant extracts is an environmentally friendly approach (Xu et al., 2006; Lestari et al., 2019; Begum et al., 2020; Ijaz et al., 2020; Almudhafar et al., 2022). Compounds found in plants that function as reducing agents in nanoparticle synthesis are terpenoids, phenolics, flavonoids, tannins, steroids, saponins, alkaloids and others (Yusof et al., 2018; Lestari et al., 2019). These compounds can be found in plants Coffee parasite (*Scurrulla ferruginea* (Roxb. Ex Jack) danser).

Coffee parasite is a parasitic plant on the coffee host that can damage the host plant. Parasite has been widely used as a traditional medicine for anti-cancer, anti-allergic, anti-tumor, medicine for flu, coughs, diarrhea, wounds, rheumatism and other degenerative diseases (Ameer et al., 2010; Marvibaigi et al., 2014; Hong et al., 2019; Roza et al., 2022a; Roza et al., 2022b). Phytochemical screening of coffee parasite leaf extract contains chemical compounds of terpenoids, alkaloids, steroids, saponins, tannins, phenols and flavonoid (Ameer et al., 2010; Marybaigi et al., 2014; Roza et al. 2022a; Roza et al., 2022b). Flavonoids are compounds that can be used as medicine for various diseases such as cancer, cardiovascular disorders and have antioxidant properties that can prevent damage caused by radicals (Roza et al., 2022a).

Previous research that in *Scurrulla ferruginea* (Roxb. Ex Jack) danser is thought to contain the flavonoid compound quercetin (Roza et al., 2023). The flavonoid compound quercetin can be used as a reducing agent in the synthesis of silver nanoparticles (Mu et al., 2019). The flavonoid compound quercetin can reduce Ag ion, where the quercetin compound which has a hydroxyl group (-OH) will be oxidized so that the hydroxyl group changes to a ketone group (C=O) as a result of the release of hydrogen atoms. After the Ag<sup>+</sup> is reduced and forms silver nanoparticles, nanoparticle growth will occur or what is called a cluster (Yusof et al., 2018; Mu et al., 2019).

## Methods

### Materials and Chemicals

The main sample is the leaves of Coffee parasite (*Scurrulla ferruginea* (Roxb. Ex Jack) danser) RG4664 from Subdistrict Sidikalang, Dairi Regency, North Sumatera. Silica gel, TLC plate, aquabides, distilled water, AgNO<sub>3</sub> (Merck), n-hexane, etil acetate and ethanol.

### Instrumentation

UV-Vis Spectrophotometry, Hot plate, Stirrer, Particle Size Analyzer, Fourier Transform Infrared Spectroscopy, analytical balance, chemical glassware.

### Separation of Secondary Metabolite Compounds

Samples of thick extracts of *Scurrulla ferruginea* (Roxb. Ex Jack) danser were analyzed using thin layer chromatography (TLC) before further separation. This TLC was carried out using a silica plate as the stationary phase, n-hexane, ethyl acetate and ethanol as the mobile phase. Eluents with different properties are used to identify the distribution of stains based on the polarity of the compounds in the sample.

The thick extract of *Scurrulla ferruginea* (Roxb. Ex Jack) was separated using Vacuum Liquid Chromatography (VLC). A total of 20 grams of methanol extract from *Scurrulla ferruginea* (Roxb. Ex Jack) fractionated with silica gel 60 GF254 as the stationary phase with several types of eluents as the mobile phase of N-hexane: Ethyl Acetate and Ethyl acetate: ethanol with comparative variations.

The combined fraction with the same R<sub>f</sub> pattern as a result of TLC from VLC was further separated using column chromatography (CC) with a stationary phase of silica gel 60 F254 (0.040-0.063 mm) and N-Hexane: Ethyl Acetate (1:1) and ethyl acetate: methanol (1 :1). The results of the collection were analyzed using TLC to see the resulting stain pattern. If the stain pattern is similar, the fraction from CC selected and identified for further compound transformation.

### Silver nanoparticle synthesis

The solution is synthesized by using the stirrer method, where 50 ml of solution silver nitrate (AgNO<sub>3</sub>) is poured into the beaker glass, added 5 ml of extract, and then heated at a temperature of 70° C for 60 minutes while stirring with speed 1500 rpm. The same method is also used in nanoparticle synthesis using the flavonoid quercetin coffee parasite. Furthermore, each solution is characterized by using Particle Size Analyzer and Fourier Transform Infrared Spectroscopy.

## Results and Discussion

The beginning of the isolation is extraction using maceration. Some of these are used for nanoparticle synthesis and some more were isolated using chromatographic methods. Isolation with VLC done for helps reduce the density of spots or bands formed in the GCC and makes it easier to interpret column results. The polar fraction from the VLC results was separated again using the gravity column chromatography (GCC) method. Separation with GCC uses samples from the polar fraction of KVC with a retention time close to quercetin based on TLC. Test 3 eluents to determine the level of purity of the compounds. The results of the 3 eluent tests that have been carried out are then compared with the standard quercetin standard to see the similarity of the stain spots using TLC (Juwitaningsih et al., 2022). The similarity of stain spot and time value retention in Fig.-1 isolate which is believed as quercetin used for the next nanoparticle synthesis.

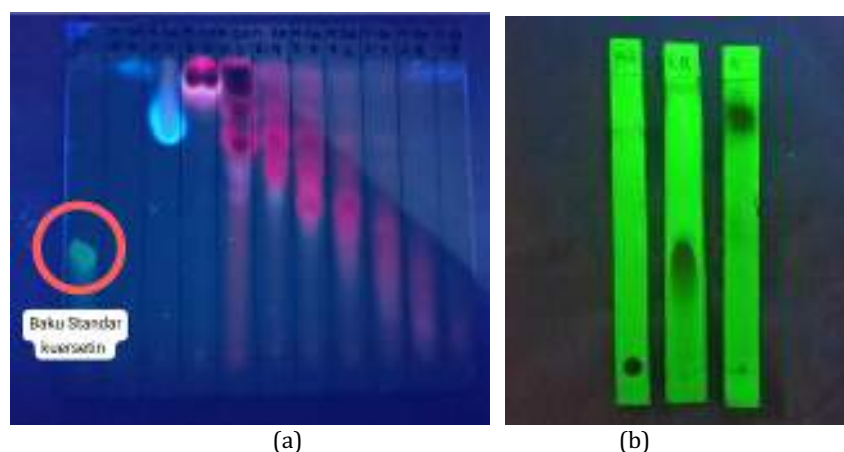


Fig-1. (a) TLC fractionation of extract coffee parasite (b) quercetin isolate

**Synthesis Silver Nanoparticle Particles Using Extract coffee parasite**

Silver nanoparticles synthesized use a reduction method which is environment friendly, because it uses natural plant coffee parasite as a reductor. Metabolites secondary in coffee parasite are known can reduce silver nitrate ( $AgNO_3$ ) which can produce silver nanoparticles ( $Ag$ ). The color change of the synthesis silver nanoparticle ( $Ag$ ) results using extract parasite coffee can be observed in Fig-2.

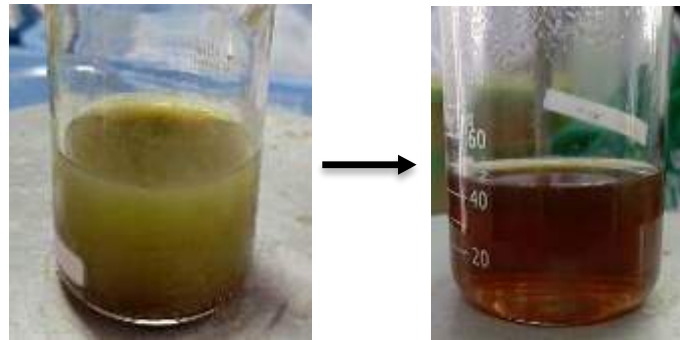


Fig-2. Synthesis silver nanoparticle ( $Ag$ ) use extract coffee parasite.

The silver nanoparticle ( $Ag$ ) formation with reduced  $AgNO_3$  is marked with a color change that occurs consequence of bioreduction from extract leaf parasite coffee. Observation change color is done after a warmup at a temperature  $70^\circ C$  using stirrer 1500 rpm. The silver nanoparticle ( $Ag$ ) formation indicates that the  $Ag^+$  in the media reaction has changed to become  $Ag^0$ .

On the synthesis of silver nanoparticles uses extract parasite coffee 5 variations were carried out time warm-up that is for 60 minutes, 90 minutes, 120 minutes, 150 minutes, and 180 minutes to see the effect of heating up of silver nanoparticle is formed. The result shows silver nanoparticles from all variations of times on the same color which is the same dark red, aside from that there is precipitate during the synthesis process which settles at the bottom container. To know the form of precipitate silver nanoparticles then centrifuged at 8000 rpm for 45 minutes. Furthermore, the results of centrifugation were then separated using paper strain, and then dried with the oven. The results obtained that the precipitate colored is chocolate.

After observed from color change, this solution was then confirmed to have known peak uptake highest use of UV-Vis spectrophotometry on 200-700 nm wavelength. The size of the silver nanoparticle formed can predicted by seeing a long wave on peak uptake. Moreover, silver nanoparticle extracts were analyzed with Particles Size Analyzer (PSA) to know the particle size formed during process synthesis.

PSA from solution silver nanoparticle use extract parasite coffee obtained size particle that is 214 nm. This result is not in accordance with the size of nanoparticles that are between 1-100 nm as can be seen in Fig-3. Size which is not in accordance with allegedly caused by the content of other compounds which is not dissolved in the process of synthesis in the content of extract coffee parasites stick with particles silver is formed so that formed bigger particle size. The resulting size it's not the original size of silver particles yet the compound size of non polar which covers silver particle.

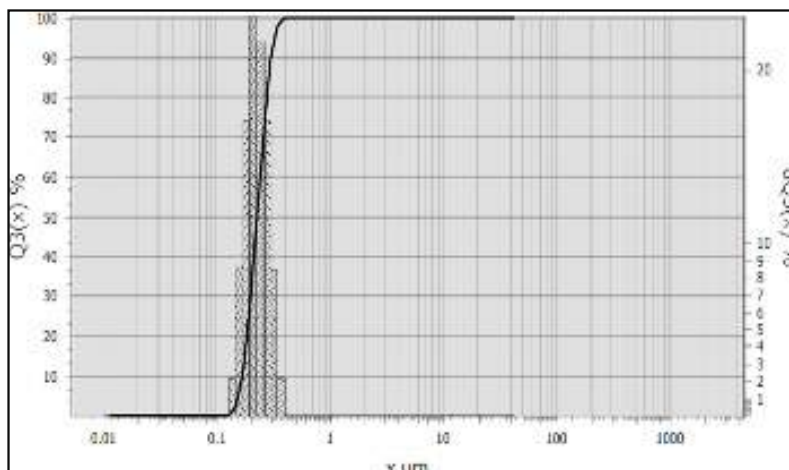


Fig-3. Size particle from synthesis silver nanoparticle using extract coffee parasite

**Synthesis Silver ( $Ag$ ) nanoparticle using Flavonoid Isolate of Coffee Parasite**

Silver nanoparticle were synthesized using flavonoids quercetin with the same treatment as the extract. The flavonoid quercetin is known as a reductor in the synthesis silver nanoparticle. Synthesis of silver nanoparticle using flavonoids quercetin change color that very different compared to with the extract. On flavonoids, quercetin happens to change that color significantly. When flavonoids quercetin solution were added into the  $AgNO_3$  solution formed into color purple light, this color change signifies that reduction happened. However, this color was not last, the purple bright color slowly faded producing clear yellow. As seen in Fig-4 this color still endures until the synthesis process is finished.

The color change from bright purple become clear yellow showed the silver ion reduction process occurs that forms silver nanoparticle. However, silver nanoparticle solution produced from synthesis using flavonoid quercetin provides low color stability. The instability of silver nanoparticle solution is caused by a number of factors, like concentration, temperature, stirring, and time reaction. Furthermore, required analysis to know the size particle silver formed, to know size particles formed, therefore, silver nanoparticle solution using flavonoids quercetin was analyzed using instruments PSAs.

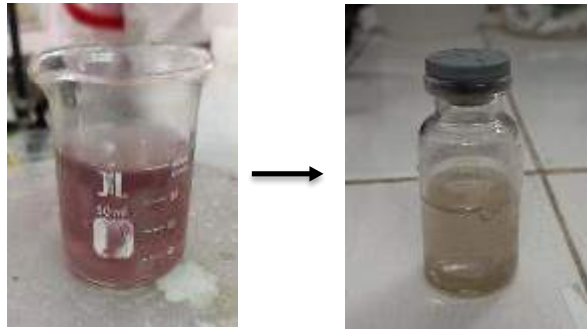


Fig-4. Color change of synthesis silver nanoparticle using quercetin.

Silver nanoparticle solution using the flavonoid quercetin done variations in storage period. In Fig-5 the first solution had done storage period of 5 days before being analyzed using PSA instrument, second solution with no storage period, means direct analyzed after completion of the synthesis process. This thing aims to know the influence of time storage on particles size formed.

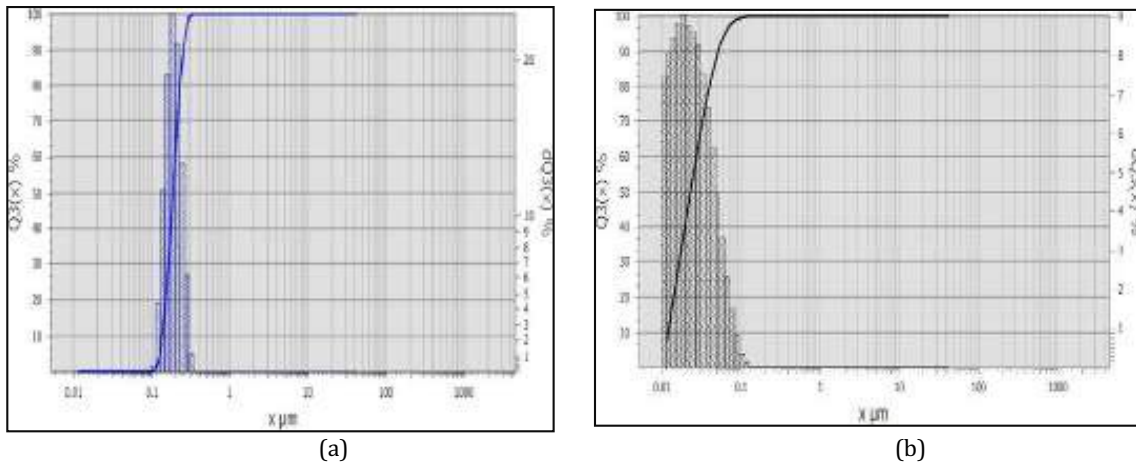


Fig-5. (a) Size particle with period storage 5 day (b) Size particle without storage period.

On sample analysis from silver nanoparticle solution using the flavonoid quercetin obtained size particles by using a storage period is 401 nm and solution without time storage is 29 nm. The particle size has an effect on time storage to the size particle produced. This is because silver nanoparticle solution is unstable so the trend for agglomeration and form bigger particle size. Interaction of interparticle can cause silver particles stick each other and shape bigger particles. Moreover, environmental condition such as temperature, humidity, and pH can also influence the stability of particle silver and cause agglomeration. Using a surfactant or stabilizer can help prevent agglomeration, but its effectiveness can be reduced along with time storage (Khan et al., 2019).

FTIR used to identify compounds involved in synthesis silver nanoparticle. This thing can help ensure that the desired compound has formed. Analysis of research this carried out in silver nanoparticle solution with no storage period, because of the size particles is good.

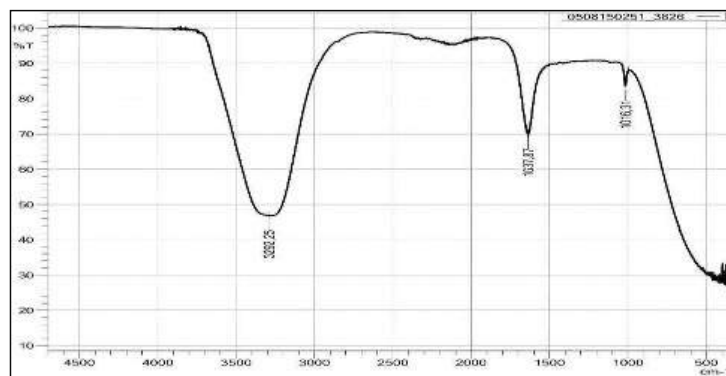


Fig-6. Spectrum IR of solution silver nanoparticle (Ag) with isolate quercetin.

From the FTIR in Fig-6, spectrum obtained a number of peak in numbers wave which different, this show that exists group functional were different. At wave number 3229.25 cm<sup>-1</sup> show vibration stretching OH groups are indicated as hydroxyl. At wave number 1637.87 cm<sup>-1</sup> show function group C=O which is indicated as ketones. Lastly, on numbers wave 1016.31 cm<sup>-1</sup> show function group CO Which is indicated as alcohol (Coates et al., 2006).

**Antioxidant Test Silver Nanoparticle (Ag) Coffee Parasite**

Antioxidant testing usually use DPPH method (2,2-diphenyl-1-picrylhydrazyl). DPPH is a radical which stable and used as antioxidant activity of extract plants (Roza et al., 2022a). The results of antioxidants activity in silver nanoparticle using extract leaf coffee parasite and silver nanoparticle uses the flavonoid quercetin can be seen in Fig-7 and Fig-8.

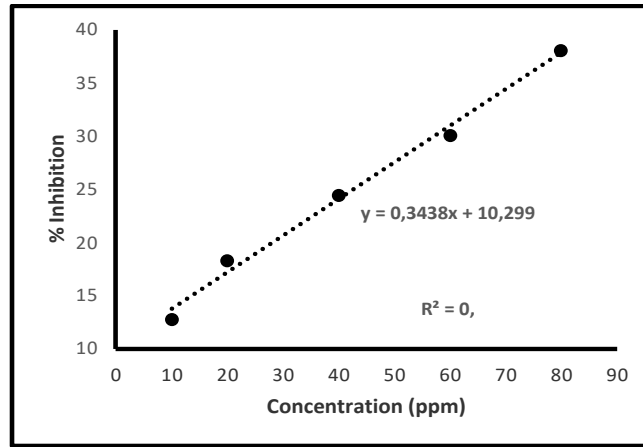


Fig-7. Activity diagram antioxidant silver nanoparticles and extract parasite coffee.

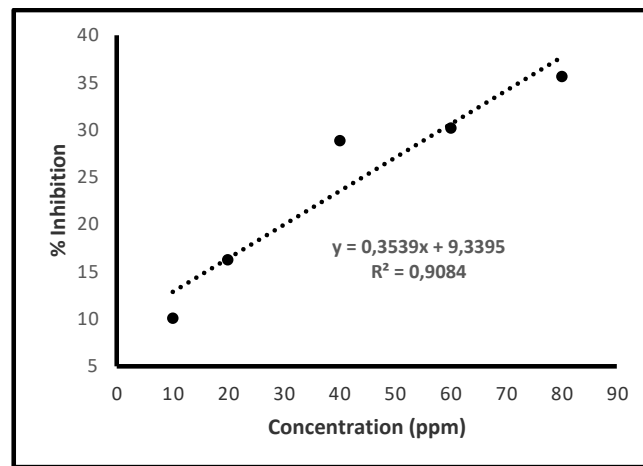


Fig-8. Activity diagram antioxidant silver nanoparticles with quercetin.

The figures show that as the concentration of the sample (µg/ml) grew, so did the percentage of inhibition. Based on the linear regression, the price of price of IC<sub>50</sub> can be use as a parameter for state the antioxidant activity. IC<sub>50</sub> shows a capable of concentration reducing 50% of radicals DPPH free (Molyneux, 2004). Strength level antioxidant is very strong when IC<sub>50</sub> value < 50 µg / mL; strong when IC<sub>50</sub> value 50-100 µg /mL; moderate when IC<sub>50</sub> 101-150 µg /mL; and weak when IC<sub>50</sub> > 150 µg / mL. The smaller the IC<sub>50</sub>, the bigger the power of the dampening (Ozgen et al., 2016). Based on the results study obtained IC<sub>50</sub> extract leaf coffee parasite is 115.4 which is included level moderate as antioxidants. The result in antioxidant nanoparticles with quercetin is 114.9. Quercetin is a purer flavonoid, therefore it is more effective in reducing silver ions into silver metal nanoparticles.

**Anticancer Test of Silver Nano Particles-Coffee Parasite**

Overall, it has been widely reported that Silver Nanoparticles can activate cytotoxicity in cancer cells and inhibit tumor development without killing normal cells. However, the results of anticancer tests using silver nanoparticles with coffee parasite flavonoid isolates against MCF-7 and HeLa cancer cells showed insignificant results as can be seen on Table 1.

The curve of the relationship between sample concentration and percent cell viability is generally for a good anticancer, the higher the concentration, the lower the percentage of cancer cell viability. Although the test results on HeLa cervical cancer cells and MCF-7 breast cancer cells, the results of the synthesis of silver nanoparticles both with extract and with quercetin isolate in the study did not show a significant decrease. The curves from the two cancer cell tests also show the same phenomenon, namely that the synthesis with the flavonoid isolate quercetin is better than the extract. Besides that, the addition of Tween 80 as a stabilizer also has an effect on reducing the percentage of cancer cell viability. Although morphology does not show strong differences.

Table 1. Anticancer test table for synthesis of silver nanoparticles with coffee parasite.

| Test cell | Silver nanoparticle synthesis | Concentration vs Viability | Morphology on concentration 1000 µg/ml | % living concentration 1000 µg/ml |
|-----------|-------------------------------|----------------------------|--|-----------------------------------|
| MCF-7     | Parasite coffe extract        |                            |  | 112                               |
|           | Quercetin                     |                            |  | 99                                |
|           | Quercetin + Tween 80          |                            |  | 80                                |
| HeLa      | Parasite coffe extract        |                            |  | 103                               |
|           | Quercetin                     |                            |  | 88                                |
|           | Quercetin + Tween 80          |                            |  | 77                                |

Based on this test, it was found that the analyzed IC50 value was greater than 1000 µg/ml. In other words, the test compound has low toxic properties against HeLa cervical cancer cells and MCF-7 breast cancer cells. Even though the IC50

value was large at a concentration of 1000 µg/ml, in both HeLa and MCF-7 cells, the percentage of live cells in the nanoparticles with the extract was above 100%, with quercetin isolates being 88 and 99%, respectively. Meanwhile, with the addition of tween, 80 percent of the cells were alive, 77% for HeLa cells and 80% for MCF-7 cells. This difference is possible because the time span between synthesis and anticancer testing is quite long. The addition of stabilizers has not had a significant effect because the optimum concentration and type of stabilizer have not been varied in the synthesis process. A cytotoxicity study on silver nanoparticles with Tamarindus indica fruit peel extract reported that the dose-dependent toxicity effect against breast cancer cells (MCF-7) using MTT assay, the inhibitory concentration (IC<sub>50</sub>) was found to be 20µg/ml and its anticancer potential was discovered using live and dead assay (Ao/EtBr), ROS and Rho123 assay. In other words Silver nanoparticles from Tamarindus indica fruit peel extract may be a potential therapeutic agent for the treatment of human breast cancer.

## Conclusion

Synthesis of silver nanoparticles using the flavonoid quercetin with a storage period of 5 days produced a particle size of 401 nm and a solution without a storage period produced a particle size of 29 nm. PSA results on synthesis of silver nanoparticle with abstract leaf coffee parasite size of 214 nm particles, compared with using flavonoid isolate quercetin size the of 29 nm. Antioxidant between silver nanoparticle solution using extract *Scurrulla ferruginea* (Roxb.Ex Jack) danser and the silver nanoparticle using the flavonoid quercetin obtained an IC<sub>50</sub> value 115.4 and 114.9. Respectively. which is consider on level moderate as antioxidants. Even though the nanoparticle anticancer test was very weak, overall, the synthesis of silver nanoparticles using flavonoid isolates was better than using extracts from coffee parasite leaves.

## Conflict of Interests

The author(s) declares that there is no conflict of interest in this research and manuscript.

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